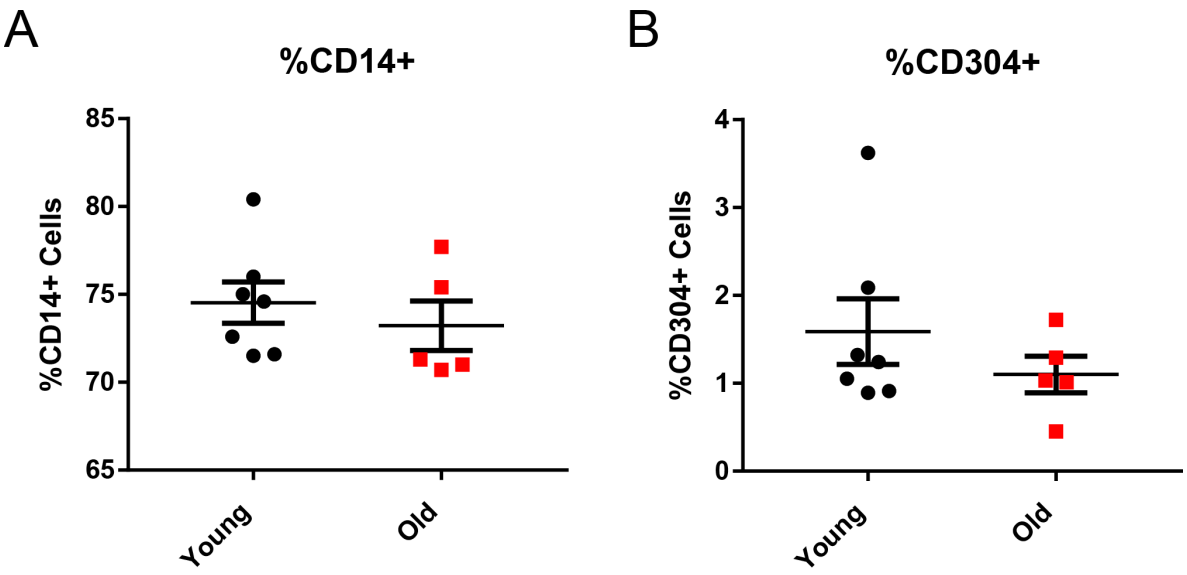

Fig. S1. Monocyte purity and plasmacytoid DC numbers are comparable between age groups. Monocytes were negatively enriched from the peripheral blood of younger (n = 7) or older (n = 5) donors. Enriched cells were then analyzed by flow cytometry to assess the frequency of (**A**) CD14+ monocytes or (**B**) CD304+ plasmacytoid dendritic cells. Data are presented as means ± SEM.

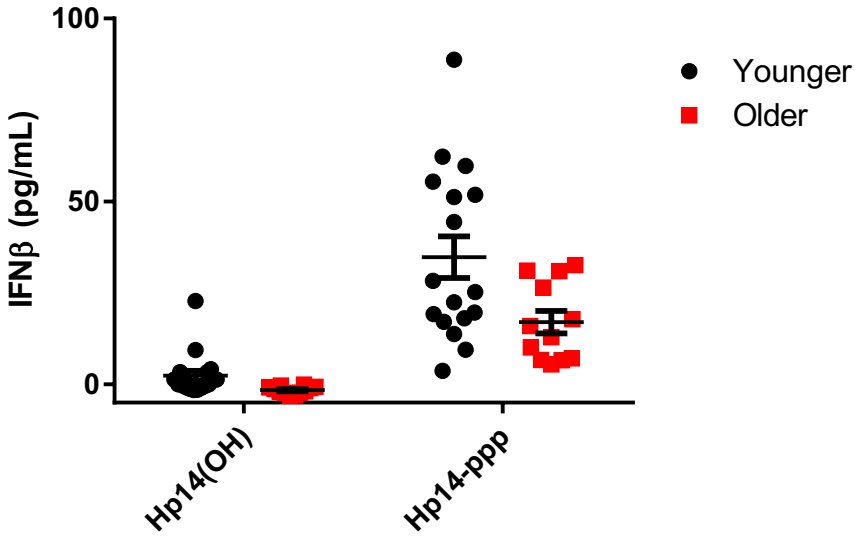

Fig. S2. IFN Induction in response to dsRNA transfection depends on a 5'-ppp motif in human monocytes.

Monocytes from younger (n = 18) or older (n = 12) human donor blood were transfected with a 14 base pair hairpin dsRNA ligand bearing either a 5'-triphosphate (Hp14ppp) or 5'-hydroxyl (Hp14(OH)) motif for 60 minutes, after which cells were incubated for an additional 11 hours. IFN-β levels were then quantified in cell supernatants. Data are presented as means ± SEM.

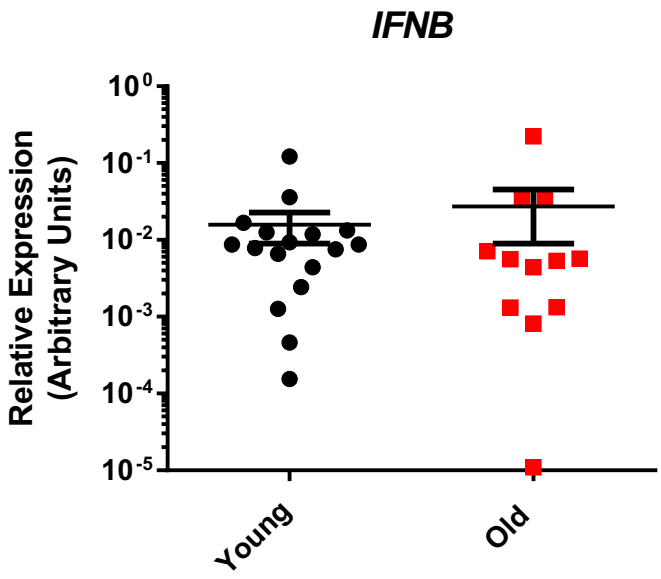

Fig. S3. Basal *IFNB* expression is comparable in human monocytes from older and younger donors.

Monocytes from younger (n = 18) or older (n = 12) human donor blood were isolated and IFNB expression was quantified by qPCR in the absence of stimulation. Data are presented as means ± SEM.

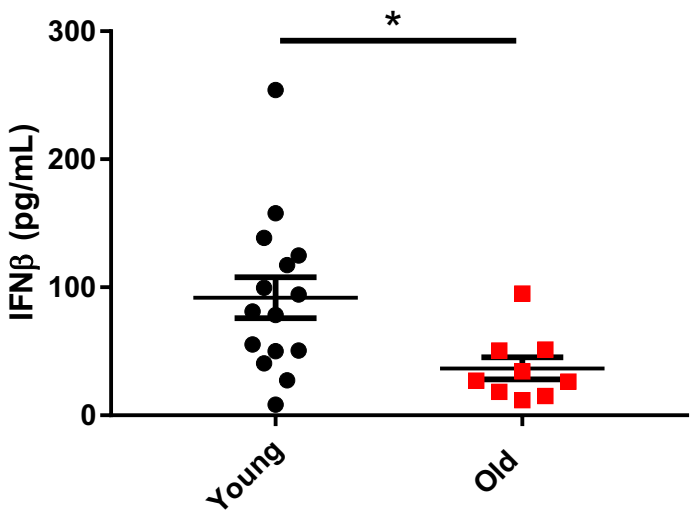

Fig. S4. IFN-β pretreatment is insufficient to ablate age-related differences in IFN-β secretion upon RIG-I stimulation.

Monocytes from younger (n = 15) or older (n = 9) human donor blood were left untreated, or were treated for 4 hours with 10 units of recombinant human IFN-β. Cells were then transfected with a RIG-I specific ligand for 60 minutes, after which cells were incubated for an additional 11 hours. IFN-β levels were then quantified in cell supernatants. *P<0.01; Student t-test.

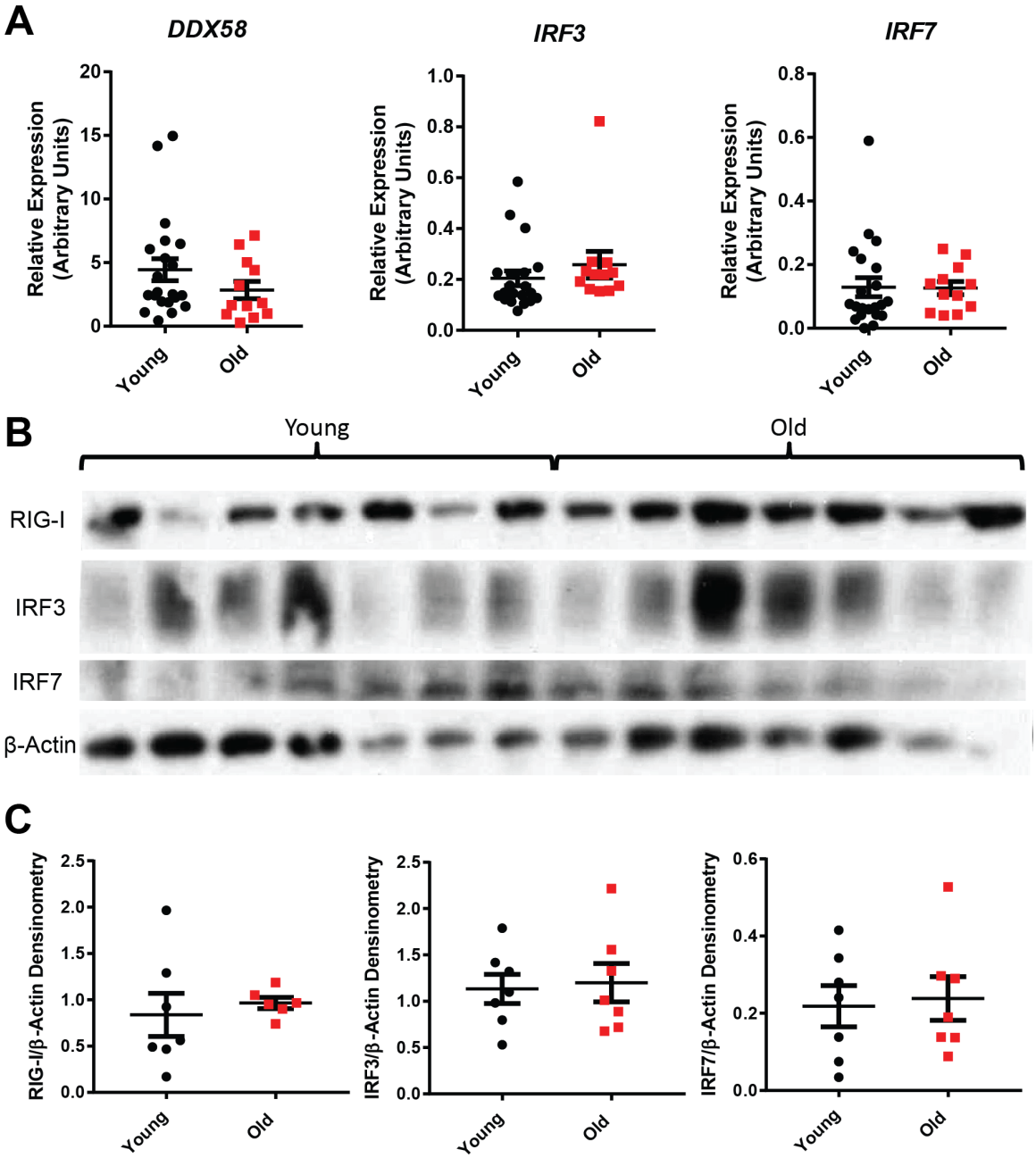

Fig. S5. Basal levels of RIG-I, IRF3, and IRF7 protein are comparable in older and younger monocytes.

(**A**) Basal expression of *DDX58* (RIG-I), *IRF3*, and *IRF7* was measured in monocytes from younger (n=18) or older (n=12) donors by qPCR. (**B**) Monocytes from younger (n = 7) or older (n = 7) human donor blood were isolated and total protein was immediately isolated from unstimulated cells. Levels of β-actin, RIG-I, IRF3, and IRF7 were assessed by western immunoblot. (**C**) Densitometry of each lane was measured using ImageJ, and for each donor RIG-I, IRF3, and IRF7 levels were normalized to β-actin levels. Data are representative of three independent Western blots, and are presented as means ± SEM.

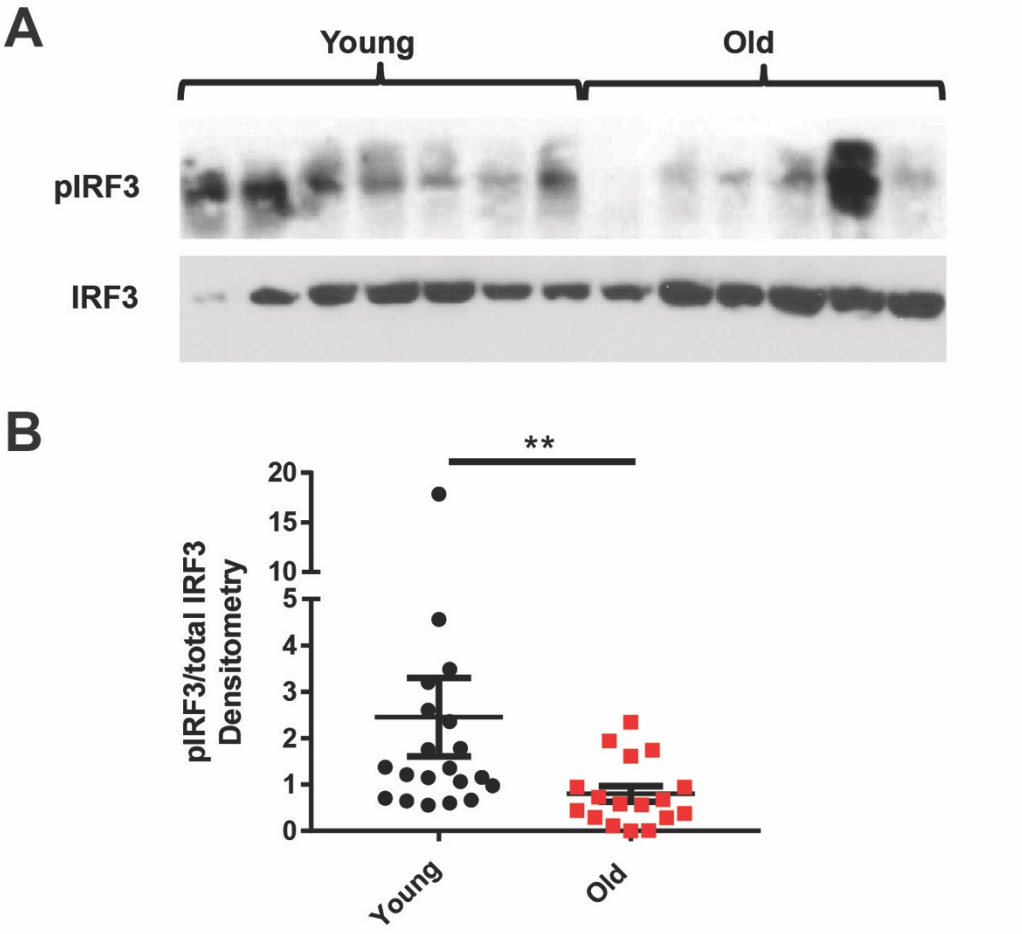

Fig. S6. IRF3 phosphorylation is impaired in monocytes from older donors.

(**A**) Monocytes from younger (n = 7) or older (n = 6) human donor blood were transfected with a RIG-I specific ligand for 60 minutes, after which time total protein was isolated. Levels of β-actin and phospho-IRF3 were assessed by western immunoblot. (**B**) Densitometry of each lane was measured using ImageJ, and for each donor phospho-IRF3 levels were normalized to β-actin levels. These results are pooled from three independent blots. **P<0.01; Mann-Whitney test.

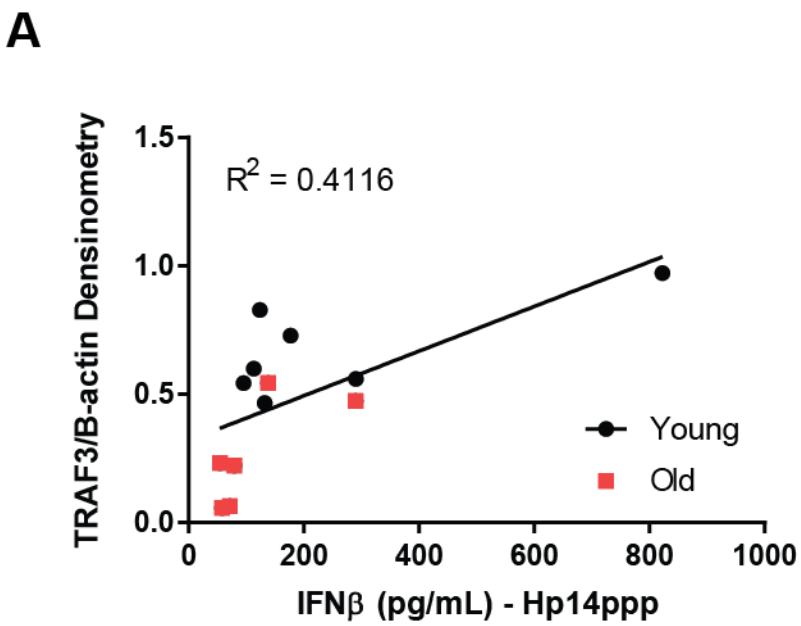

Fig. S7. Basal TRAF3 protein levels are positively correlated with IFNβ secretion in response to RIG-I stimulation.

Monocytes from the same donors that were used to measure basal TRAF3 protein levels in Figure 3B were stimulated with a RIG-I specific ligand for 12 hours, after which time supernatant IFNβ levels were quantified by ELISA. For each donor, basal TRAF3 densitometry from Figure 3B was graphed against secreted IFNβ levels upon RIG-I stimulation. R^2 is the square of Pearson's *r*, which was used assess the linear correlation between these variables.

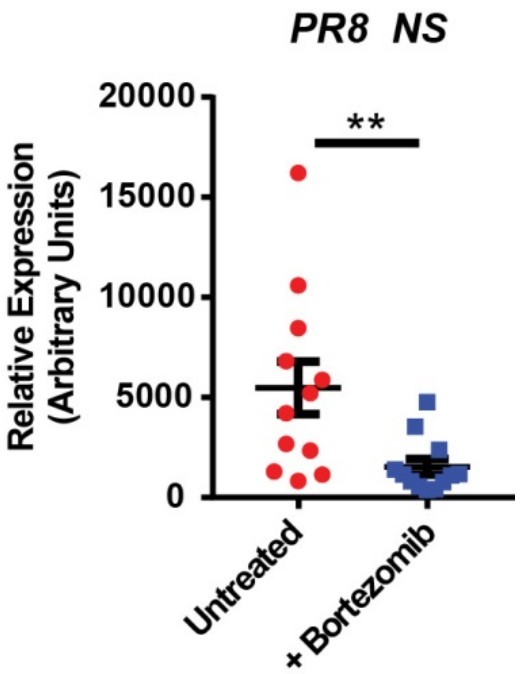

Fig. S8. Bortezomib improves antiviral responses in monocytes from older donors.

Monocytes from older donors (n = 12) were left untreated or were treated with bortezomib for 4 hr and were infected with PR8 IAV (MOI=10) for 12 hours, after which PR8 NS expression was quantified by qPCR. Data are presented as means ± SEM.,** P<0.01; Paired or Unpaired Student t-test.

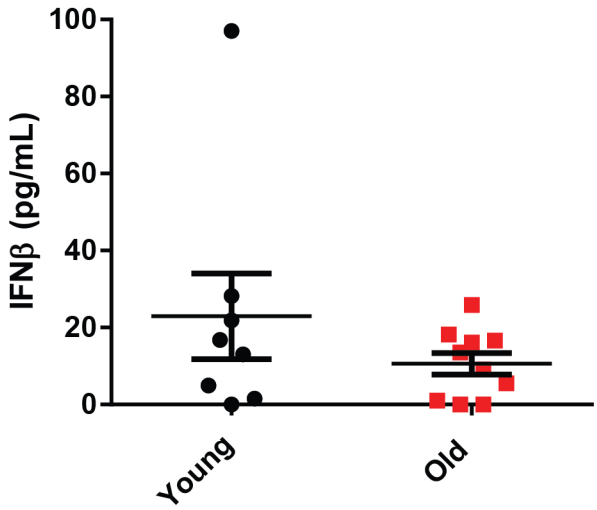

Fig. S9. Lack of age-related differences in IFN-β secretion in response to cGAMP stimulation of human monocytes.

Monocytes from younger (n = 8) and older (n = 10) human donor blood were transfected with cGAMP for a period of 60 minutes, followed by an additional 11 hour incubation period after which IFN-β levels in cell supernatants were quantified by ELISA.

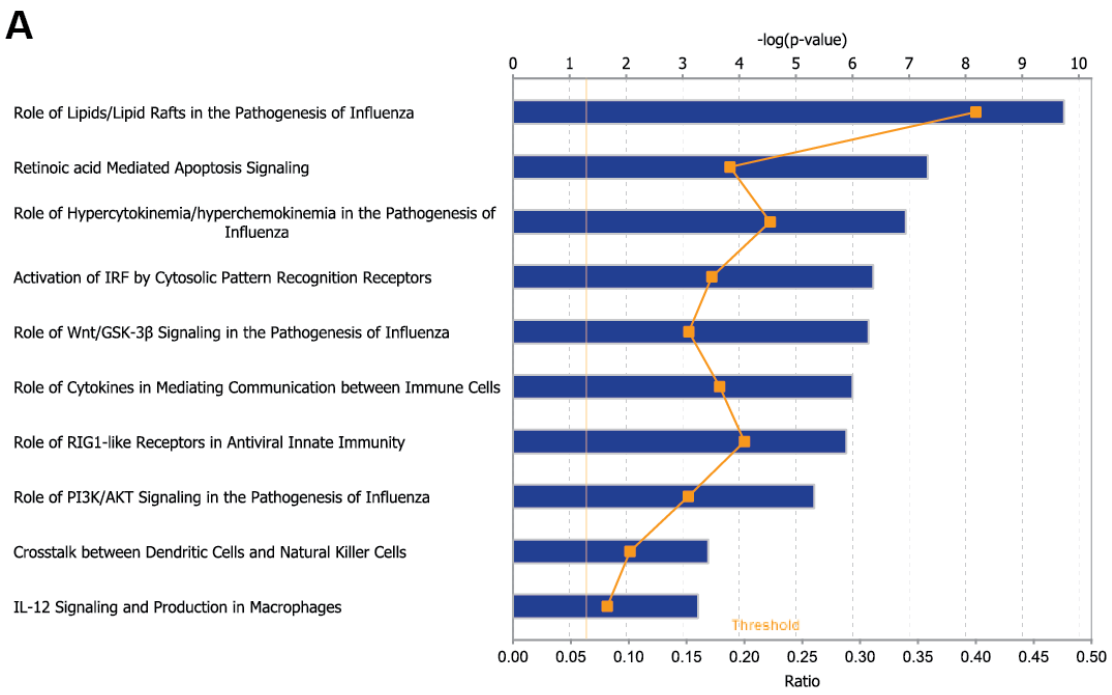

Fig. S10. RNA-seq highlights differential regulation of RIG-I, IRF, and IAV-related signaling pathways in older RIG-I–stimulated monocytes.

Ingenuity Pathway Analysis software was used to identify differentially regulated gene networks in the older and younger monocytes used for RNA sequencing in Figure 4. Differentially regulated genes with a p value <0.05 were used to identify the most differentially regulated gene networks and pathways.

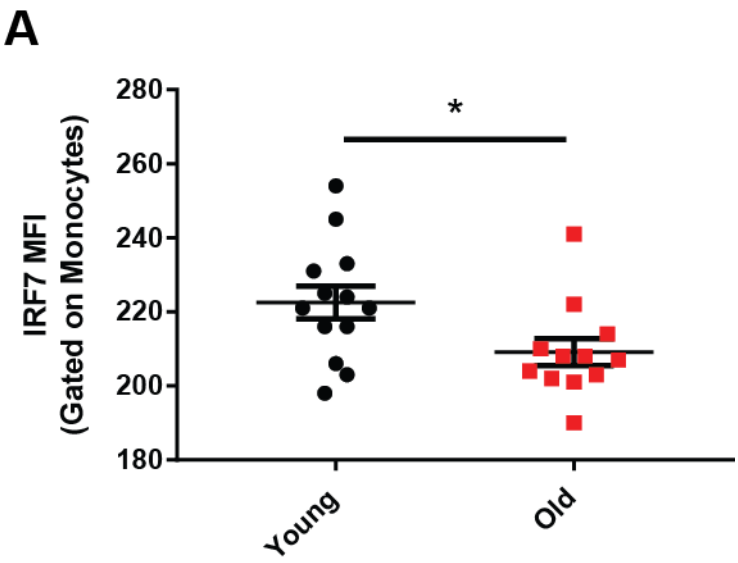

Fig. S11. Amplification-stage increase in IRF7 protein is defective in older RIG-I–stimulated monocytes.

Monocytes from younger (n = 13) or older (n = 12) human donors were stimulated with a RIG-I specific ligand for 6 hours. Cells were then fixed, permeabilized, and total IRF7 protein levels were assessed by flow cytometry and quantified based on MFI. Data are presented as means ± SEM. *P<0.05; Student t-test.

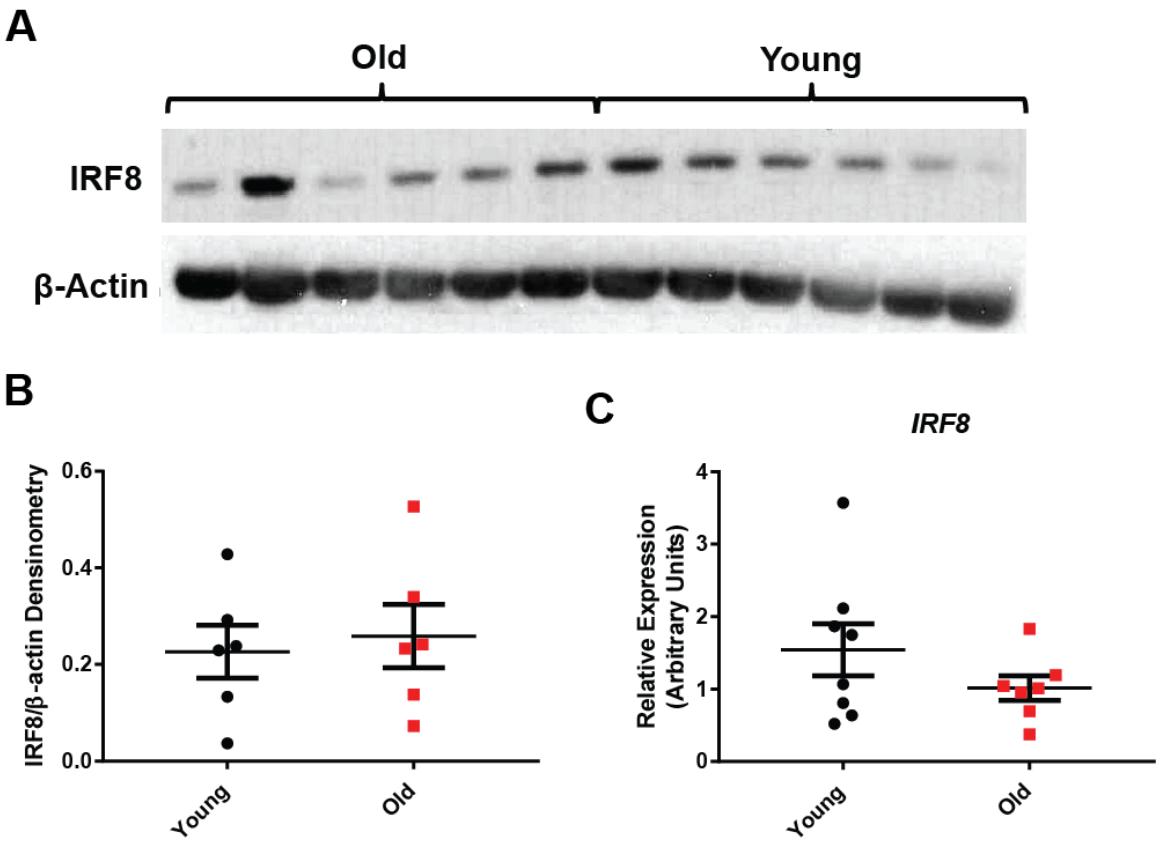

Fig. S12. Basal IRF8 protein and RNA levels are comparable in old and young monocytes.
(**A**) Monocytes from younger (n = 6) or older (n = 6) human donor blood were isolated and total protein was immediately isolated from unstimulated cells. Levels of β-actin and IRF8 were assessed by western immunoblot. (**B**) Densitometry of each lane was measured using ImageJ, and for each donor RIG-I, IRF3, and IRF7 levels were normalized to β-actin levels. (**C**) RNA was isolated from younger (n = 8) or older (n = 7) unstimulated monocytes, and *IRF8* levels were measured by qPCR. Data are presented as means ± SEM.

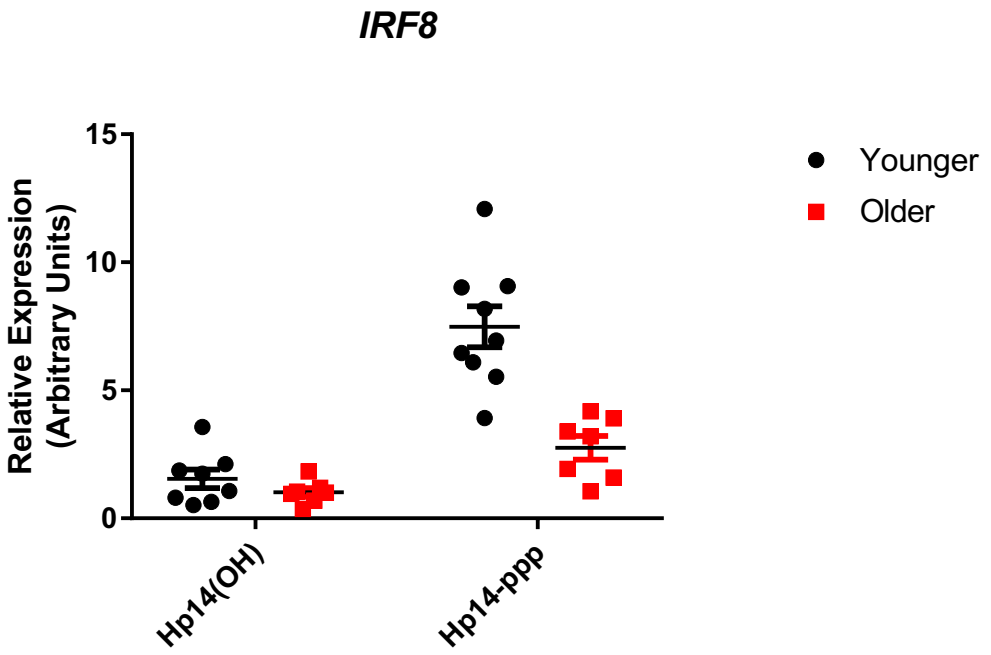

Fig. S13. Inducible *IRF8* expression is age-dependent.

Monocytes from younger (n = 9) or older (n = 7) human donor blood were transfected with a 14 base pair hairpin dsRNA ligand bearing either a 5'-triphosphate (Hp14ppp) or 5'-hydroxyl (Hp14(OH)) motif for 60 minutes, after which cells were incubated for an additional 5 hours. RNA was then isolated from cells and *IRF8* levels were measured by qPCR.

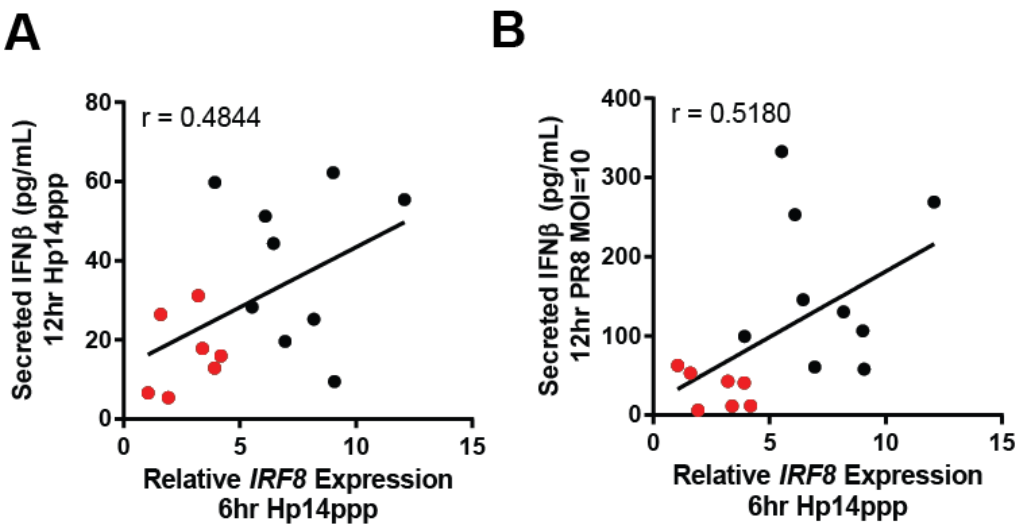

Fig. S14. Inducible IRF8 levels are positively correlated with RIG-I–inducible IFNβ secretion.

All graphs in this figure utilize the same samples as in Figure 5A-B. IFNβ levels in cell supernatants of RIG-I stimulated or PR8 IAV (MOI = 10) infected monocytes from young and old donors were measured by ELISA 12 hours post-stimulation. For each donor, *IRF8* induction at 6 hours post RIG-I stimulation were then graphed against secreted IFNβ levels upon (**A**) RIG-I stimulation or (**B**) PR8 infection. Pearson's *r* was used assess the linear correlation between these variables.

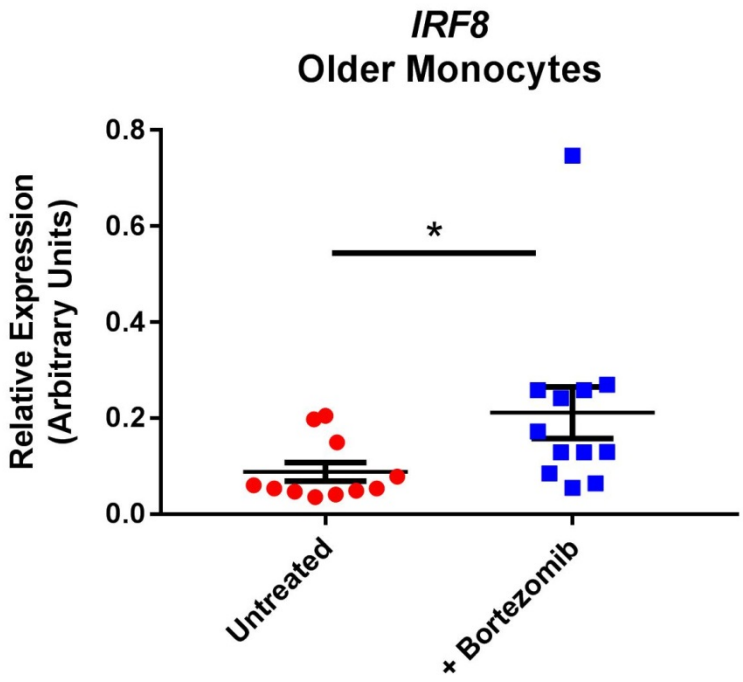

Fig. S15. Bortezomib treatment of older monocytes enhances *IRF8* induction upon RIG-I stimulation.

Monocytes from older donors (n=12) were pretreated with bortezomib for 4 hours prior to transfection with a RIG-I specific ligand for 6 hours, after which time *IRF8* expression was quantified by qPCR., Data are presented as means ± SEM. * P<0.05, Paired Student t-test.

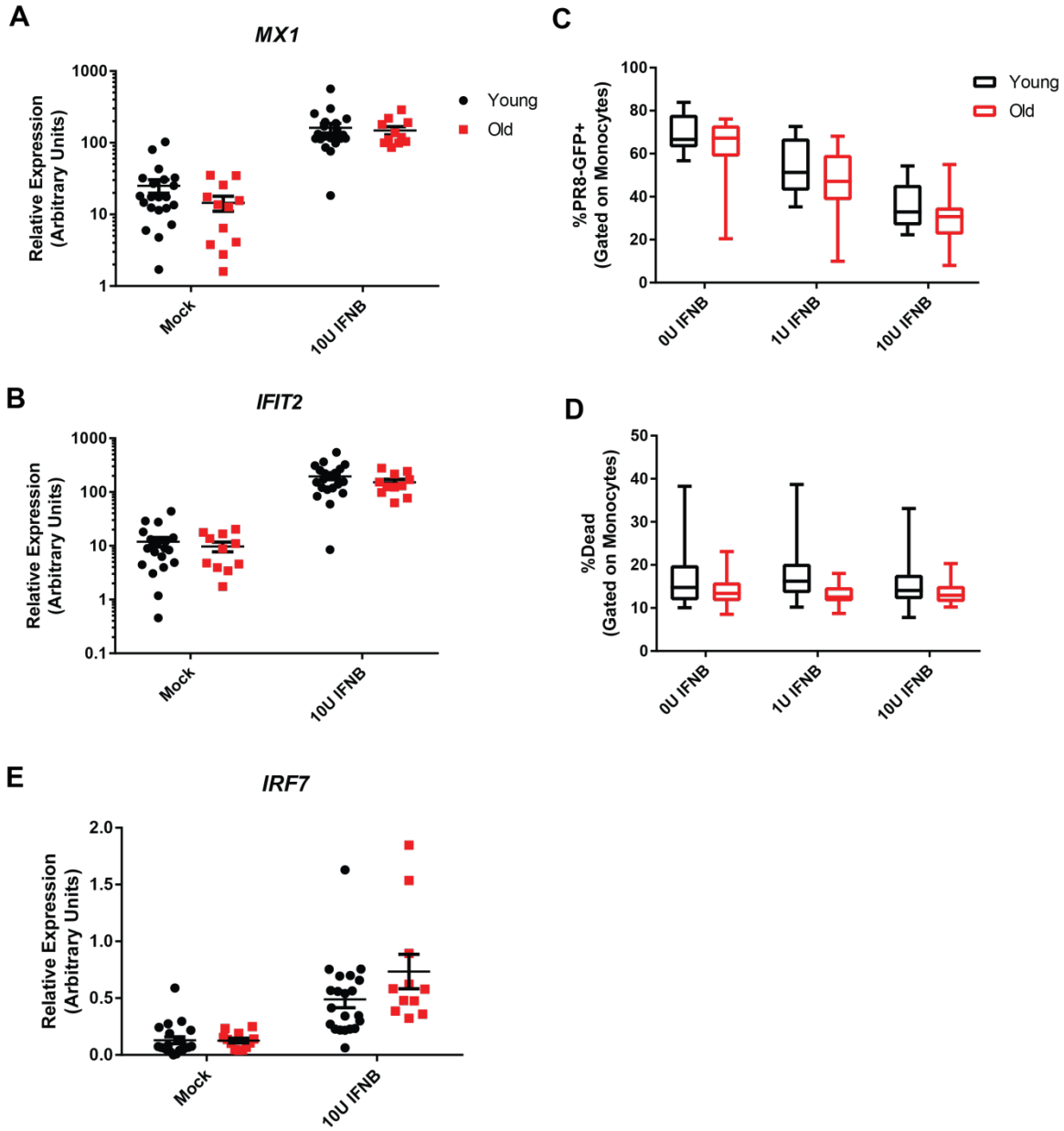

Fig. S16. IFNβ-stimulated gene induction and inducible protection from influenza is preserved with age in human monocytes.

Monocytes from younger (n = 21) and older (n = 12) human donor blood were left untreated, or were treated for 4 hours with either 1 or 10 units of recombinant human IFNβ. RNA was then isolated from cells and qPCR was used to examine expression levels of (**A**) *MX1* or (**B**) *IFIT2*. (**C-D**) Alternatively, after IFNβ treatment cells were infected with PR8-GFP IAV (MOI = 10) for 16 hours, stained for cell death, and assessed by flow cytometry to measure the frequency of PR8-GFP+ cells and of cell death. (**E**) *IRF7* induction was assessed as in A-B. Data are presented as means ± SEM.

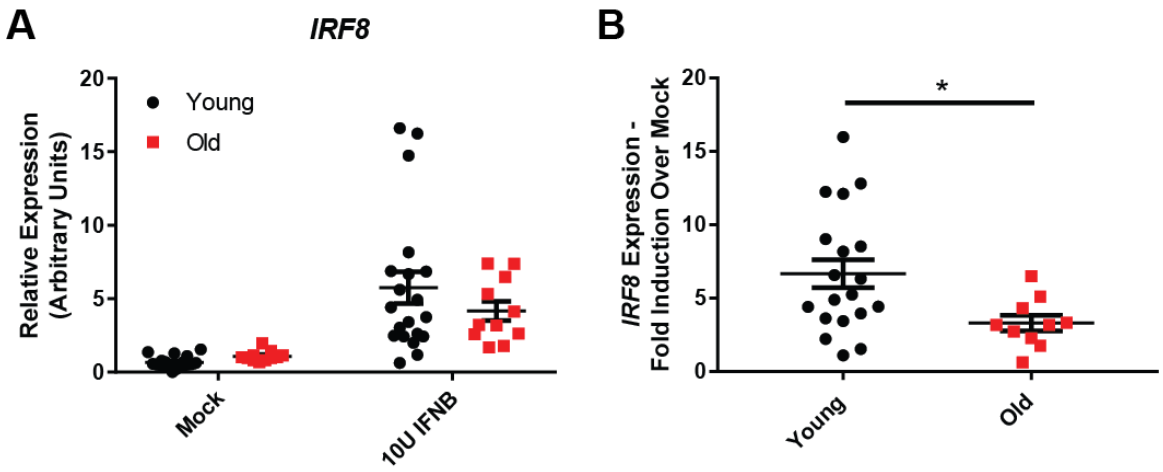

Fig. S17. *IRF8* is an interferon-inducible gene, and its induction is impaired in older monocytes.

(**A**) Monocytes from younger (n = 21) and older (n = 12) human donor blood were left untreated, or were treated for 4 hours with 10 units of recombinant human IFN-β (rIFN- β). RNA was then isolated from cells and qPCR was used to examine expression levels of *IRF8*. (**B**) For each donor, *IRF8* expression after rIFN-β treatment was normalized to expression levels in untreated monocytes to establish the fold induction of *IRF8* in these donors. Data are presented as means ± SEM. *P<0.05; Student t-test.

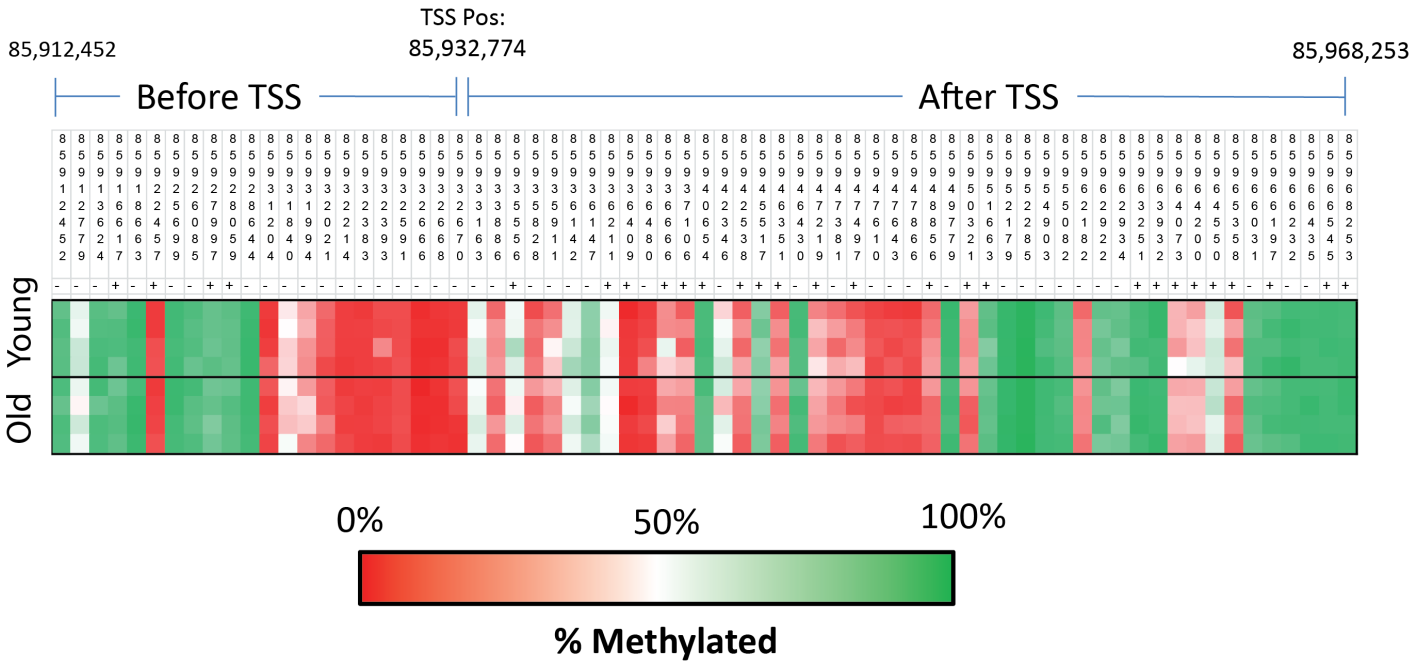

Fig. S18. Lack of age-related difference in CpG DNA methylation of the *IRF8* gene in human monocytes.

Monocytes from younger (n = 4) or older (n = 4) human donor blood were isolated and DNA was prepared from unstimulated cells. DNA was then used for DNA methylation profiling using the Infinium Methylation EPIC array platform (Illumina), which covers 860,000 CpG residues. The methylation status of *IRF8*-associated residues was arranged into a heat map using Microsoft Excel, with each row representing one donor and each column representing a particular CpG residue.

Table S1. Human Donor Characteristics

Patient Characteristics (N=148)	Young (N=88)	Older (N=60)	P-value[a]
Age (years) range (22-89), mean (SD)	26.2 (6.2)	71.4 (5.8)	<0.001
Female, n (%)	66 (75.0)	24 (40.0)	<0.001
White Race, n (%)	50 (56.8)	56 (93.3)	<0.001
Number of Comorbid Conditions, mean (SD)	1.0 (1.2)	2.7 (1.6)	<0.001
Myocardial Infarction, n (%)	0 (0.0)	1 (1.7)	0.446
Congestive Heart Failure, n (%)	0 (0.0)	0 (0.0)	--
Coronary Artery Disease, n (%)	0 (0.0)	7 (11.7)	0.003
Cardiac Arrhythmia, n (%)	2 (2.3)	5 (8.3)	0.241
Hypertension, n (%)	3 (3.4)	34 (56.7)	<0.001
Peripheral Vascular Disease, n (%)	2 (2.3)	5 (8.3)	0.241
Chronic Obstructive Pulmonary Disease, n (%)	0 (0.0)	1 (1.7)	0.446
Peptic Ulcer Disease, n (%)	2 (2.3)	3 (5.0)	0.656
Number of Prescription Medications, mean (SD)	0.7 (1.3)	3.3 (2.7)	<0.001
Statin Medications, n (%)	0 (0.0)	28 (46.7)	<0.001
Number of OTC Medications, mean (SD)	1.3 (1.5)	3.0 (1.6)	<0.001
Aspirin, n (%)	1 (1.1)	37 (61.7)	<0.001

[a]Probability for Student t-test for continuous measures, Fisher Exact test for categorical measures.

Table S2. Differences in IFN Expression in RNA-Seq Data from RIG-I Stimulated Older and Younger Monocytes

Name	log2FoldChange	p value
IFNL1	-3.02419	1.44E-11
IFNA13	-1.76628	0.000485
IFNW1	-1.68768	0.001999
IFNA2	-1.68644	0.001241
IFNA6	-1.65072	0.003402
IFNA8	-1.62166	0.003896
IFNA17	-1.61921	0.004819
IFNA1	-1.61011	0.000529
IFNA16	-1.58472	0.005082
IFNA14	-1.56731	0.000793
IFNA21	-1.47079	0.010695
IFNB1	-1.14785	0.022974
IFNE	-0.8881	0.121854
IFNA5	-0.70456	0.124738

Table S3. Differences in IRF Expression in RNA-Seq Data from RIG-I Stimulated Older and Younger Monocytes

Name	log2FoldChange	p value
IRF8	-1.41637	0.000146
IRF4	-1.10089	0.005778
IRF1	-0.40661	0.238159
IRF7	-0.41064	0.238477
IRF5	0.41349	0.287356
IRF9	0.241819	0.604984
IRF2	0.078738	0.831375
IRF3	-0.08607	0.859436
IRF6	0.209473	NA